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Effect of Environmental Stilbestrol in Shortening Prolonged Gestation in the Lactating Rat.

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Estrogen is usually administered to experimental animals by injection, inunction, subcutaneous implantation of pellets or by the oral route. Little or no attention has been paid to the possible effects of estrogen that may be present in cages in which treated animals are kept and in whose excreta quantities of hormone may be eliminated. The present report shows that minute quantities of the synthetic estrogen diethylstilbestrol, by its mere presence in the animal's cage, may reduce the gestation periods of inseminated lactating rats to normal.

The length of the gestation periods of lactating rats inseminated during the postpartum estrus may range from 23 to 40 days depending, in general, upon the number of suckling young.¹⁻⁴ The prolongation of gestation is due to delay in implantation⁵⁻⁸ and altered endocrine factors are responsible for this delay.^{2-4,9} If certain hormonal conditions are satisfied implantation will occur, even in rats suckling large litters, at the same time (day 6) as in normal, non-lactating pregnant rats. The subsequent development of such embryos is normal and parturition occurs on the 23rd day.²⁻⁴

Methods. Each of 10 female rats was placed in a separate cage with a mature male the day before parturition was expected. Immediately after delivery each litter was adjusted to 9. In all animals spermatozoa were found in the vagina within 24 hours after

parturition and the male was then removed. A pan on the bottom of the cage was filled to a depth of $\frac{1}{2}$ inch with shavings used as nesting material. In each experiment .1 mg diethylstilbestrol in oil was shaken up in 20 cc of ether and then scattered about the cage. In all cases this was done on the day that spermatozoa were found and on such other days as indicated in Table I. The cages were not cleaned until the termination of the experiment.

Vaginal smears were taken routinely. Particular attention was directed to the appearance of the "placental sign." Free blood in the vagina gave evidence of resorption of some or all of the embryos. This was always confirmed by manual palpation of the uteri.

Controls consisted of 49 untreated rats each suckling 9 young. In these, the average day of implantation was 16.9 and the average length of gestation was 32.9 days.

Results. In one female (E228d) there was no indication of implantation or pregnancy. In the remaining 9 animals implantation occurred at a time (Table I) comparable to that of a normal pregnant rat. In 5 of these animals resorption began between days 9 and 12. In 2 animals (E233a and E230d) all but one of the embryos were resorbed between days 14 and 16. In each case the remaining embryo continued to develop but was resorbed between days 22 and 26. Two animals (E227f and E227b) delivered normal litters of 7 and 5 young respectively on day 23. The young were reared and were normal. Variations in total amount of hormone scattered about the cage did not have any obvious differential effects.

Discussion. Although the hormone was scattered about the cage, its entrance into the body of the mother is undoubtedly comparable to direct oral administration. Hormone adhering to the feet or fur of the mother

¹ King, H. D., *Biol. Bull.*, 1913, **24**, 377.

² Weichert, C. K., *Anat. Rec.*, 1940, **77**, 31.

³ Weichert, C. K., *Anat. Rec. Supp.*, 1941, **81**, 28.

⁴ Weichert, C. K., *Anat. Rec.*, 1942, **83**, 1.

⁵ Kirkham, W. B., *Anat. Rec.*, 1916, **11**, 31.

⁶ Kirkham, W. B., *J. Exp. Zool.*, 1918, **27**, 49.

⁷ Kirkham, W. B., *Anat. Rec.*, 1916, **10**, 219.

⁸ Enzmann, E. V., Saphir, N. R., and Pincus, G., *Anat. Rec.*, 1932, **54**, 325.

⁹ Krehbiel, R. H., *Anat. Rec.*, 1941, **81**, 381.

TABLE I.
Effects of Scattering Diethylstilbestrol About the Cages of Inseminated Lactating Rats
Suckling 9 Young.

Rat	Day																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
E226	x										R	R														
E227f	x		x																				B			
E227c	x		x		x				R	R	R															
E227e	x		x		x						R	R	R	R	R	R	R									
E228d	x		x		x																					
E230b	x		x		x					R	R	R	R	R	R											
E230d	x		x		x										R							R	R	R	R	R
E230a	x		x		x		x					R	R													
E233a	x		x		x		x		x						R	R	R					R	R			
E227b	x		x		x		x		x														B			

x—.1 mg diethylstilboestrol scattered about cage.

R—Resorption of embryos taking place.

B—Normal litter born.

or to the skin of the pups would be licked off during the process of cleansing. Allen¹⁰ has shown that natural estrogens are inactivated by the liver whereas stilbestrol is not. Thus the hormone, entering the circulatory system through the portal vein, should be as effective as if administered by injection. Results show that stilbestrol is capable of substituting for natural estrogen in reducing the time of implantation in inseminated lactating rats to normal. In certain cases the embryos may develop to term. The high percentage of resorptions is not surprising since administration of estrogen in exact quantities is not possible by this method and the antagonistic effect of large doses of estrogen on pregnancy

is well known.

The study emphasizes the importance of isolating experimental animals that are being treated with stilbestrol. Other animals in the same cage may be profoundly affected and cause the investigator to draw erroneous conclusions from changes that might be observed. Cages should be cleaned thoroughly upon termination of experiments involving the use of stilbestrol.

Conclusions. Small amounts of diethylstilbestrol scattered about the cages of inseminated lactating rats suckling 9 young brings about implantation at the normal time (day 6) rather than around the 16th day as in untreated controls. The study emphasizes the importance of isolating experimental animals that are being treated with diethylstilbestrol.

¹⁰ Allen, M. J., *Proc. Am. Physiol. Soc.*, 53rd Meeting, 1941, 6.